Released by/Date Effective:

Author Name	Title	Signature/Date

Approver Name	Title	Signature/Date

1. PURPOSE

1.1. The purpose of this procedure is to describe how to transfect HPV plasmid DNA coding for empty capsid into HEK293TT cells to produce virus-like particles (VLPs).

2. SCOPE

2.1. This procedure applies to the HPV Serology Laboratory located at the Advanced Technology Research Facility, Room C2007.

2.2. This procedure will include the transfection of plasmid DNA into the HEK293TT cell line, VLP production, maturation, and purification of VLPs via a density-based gradient.

3. REFERENCES

3.1. HSL_LAB_005.01: HEK293TT Transfection Form, Day 1-4

3.2. HSL_LAB_005.02: HEK293TT Transfection Form, Day 5

3.3. HSL_GL_001: Waste Disposal at the Advanced Technology Research Facility

3.4. HSL_GL_002: Equipment Qualification and Calibration in the HPV Serology Laboratory

3.5. HSL_GL_003: Good Documentation Practices for the HPV Serology Laboratory

3.6. HSL_GL_004: Laboratory Notebook Control and Use for the HPV Serology Laboratory

3.7. HSL_GL_006: Reagent Preparation for the HPV Serology Laboratory

3.8. HSL_GL_007: Reagent and Chemical Expiry in the HPV Serology Laboratory

3.9. HSL_GL_008: Laboratory Flow and Gowning Procedures for the HPV Serology Laboratory

3.10. HSL_GL_009: HPV Serology Laboratory BSL-2 Procedures

3.11. HSL_GL_010: Control and Request of Documents in the HPV Serology Laboratory

3.12. HSL_EQ_001: Biosafety Cabinet (BSC) Use and Maintenance

3.13. HSL_EQ_002: Operation, Use and Maintenance of C02 Incubators

3.14. HSL_EQ_003: Use and Maintenance of the Thermo Fisher Sorvall Legend XTR Centrifuge in the HPV Serology Laboratory

3.15. HSL_EQ_006: Use and Maintenance of the Cellometer Auto 2000

3.16. HSL_EQ_007: Use and Maintenance of a Refrigerator in the HPV Serology Laboratory

3.17. HSL_EQ_008: Use and Maintenance of -80°C Freezers in the HPV Serology Laboratory

3.18. HSL_EQ_009: Use and Maintenance of the Liquid Nitrogen Freezer

3.19. HSL_EQ_012: Use and Maintenance of Pipettes in the HPV Serology Laboratory

3.20. HSL_EQ_015: Use and Maintenance of an Analytical & Precision Balance

3.21. HSL_EQ_016: Use and Maintenance of -20°C Freezer in the HPV Serology Laboratory

3.22. HSL_EQ_018: Use and Maintenance of an Inverted Microscope

3.23. HSL_EQ_021: Use and Maintenance of Nanodrop 1000 Spectrophotometer

3.24. HSL_EQ_024: Use and Maintenance of the Optima XPN Ultracentrifuge System

3.25. HSL_LAB_01: 293TT Cell Culturing and Maintenance

4. RESPONSIBILITIES

4.1. The Research Associate, hereafter referred to as analyst, is responsible for reviewing and following this procedure.

4.2. The Scientific Manager or designee is responsible for training personnel in this procedure and reviewing associated documentation.

4.3. The Quality Assurance Specialist is responsible for quality oversight and approval of this procedure.

5. REAGENTS, CHEMICALS AND EQUIPMENT

5.1. Reagents

5.1.1. 10% Brij58 (HSL_GL_006: Section 23)

5.1.2. 1M Ammonium Sulfate (HSL_GL_006: Section 30)

5.1.3. 27% OptiPrep (HSL_GL_006: Section 26)

5.1.4. 293TT VLP/PsV Transfection cell culture media (DMEM 10A) (HSL_GL_006: Section 21)

5.1.5. 33% OptiPrep (HSL_GL_006: Section 27)

5.1.6. 39% OptiPrep (HSL_GL_006: Section 28)

5.1.7. 5M NaCl (KD Medical, Cat # RGF-3270)

5.1.8. Benzonase (Sigma, Cat # E1014-25KU)

5.1.9. DPBS_0.8M (DPBS_0.8M) (HSL_GL_006: Section 24)

5.1.10. DPBS-MgCl2 10mM A/A (DPBS_MgCl_AA) (HSL_GL_006: Section 22)

5.1.11. Dulbecco's Phosphate-Buffered Saline (DPBS) (Life Technologies, Cat # 14190-136)

5.1.12. Expression plasmid coding HPV capsid sequences

5.1.13. HEK 293TT cells

5.1.14. Lipofectamine 2000 (Life Technologies, Cat# 11668-019)

5.1.15. Opti-MEM (Life Technologies, Cat # 11058-021)

5.1.16. PEI (HSL_GL_006: Section 34)

5.1.17. Plasmid-Safe DNase (Epicentre Biotechnologies, Cat # E3101K)

5.1.18. Transporter 5 (Polysciences, Inc., Cat # 26008-50)

5.1.19. Trypsin-EDTA 0.05% (Life Technologies, Cat # 25300-054)

5.2. Consumables

5.2.1. 10 mL serological pipets (Warehouse, Cat # 66401370 or equivalent)

5.2.2. 25 mL serological pipets (Warehouse, Cat # 66401361 or equivalent)

5.2.3. 5 mL serological pipets (Warehouse, Cat # 66401365 or equivalent)

5.2.4. 50 mL conical tubes (Warehouse, Cat # 66401493 or equivalent)

5.2.5. 50 mL serological pipets (Warehouse, Cat # 66401363 or equivalent)

5.2.6. 500 mL conical centrifuge tubes (Thomas Scientific, Cat # 8600A70 or equivalent)

5.2.7. 5-Layer Flask (VWR, Cat # 89204-478 or equivalent)

5.2.8. 8-Layer CELLdisk (Greiner Bio-One, Cat # 678108 or equivalent)

5.2.9. BD 1 mL syringe with 25-gauge needle (Warehouse, Cat # 66301465 or equivalent)

5.2.10. Corning Polystyrene Roller Bottle 2 L (VWR, Cat # 89184-640 or equivalent)

5.2.11. Media Storage Bottle 1 L (Thomas Scientific, Cat # 1743D15 or equivalent)

5.2.12. Nalgene 0.2 µM PES membrane 500 mL filter bottle (Thomas Scientific, Cat # 1234K58 or equivalent)

5.2.13. Parafilm (Warehouse, Cat # 66401356 or equivalent)

5.2.14. Siliconized 1000 µL pipet tips (Thomas Scientific, Cat # 7738E30 or equivalent)

5.2.15. Siliconized 200 µL pipet tips (Thomas Scientific, Cat # 7738E15 or equivalent)

5.2.16. T-150 Flask (Thomas Scientific, Cat # 9381J33 or equivalent)

5.2.17. T-225 Flask (Thomas Scientific, Cat # 9381M60 or equivalent)

5.2.18. Thinwall Polypropylene Tubes, 14 mL (Beckman Coulter, Cat # 331374)

5.2.19. Thinwall Polypropylene Tubes, 5 mL (Beckman Coulter, Cat # 326819)

5.3. Equipment

5.3.1. BSC

5.3.2. Cannulas (VWR, Cat # 20068-680 or equivalent)

5.3.3. Cellometer

5.3.4. Centrifuge

5.3.5. Incubator

5.3.6. Inverted Light Microscope; Nikon TMS

5.3.7. Nanodrop 1000

5.3.8. Pipettes

5.3.9. Precision Balance

5.3.10. Refrigerated micro centrifuge

5.3.11. Rotor SW40.1Ti, rated for >200,000 x g

5.3.12. Rotor SW55Ti, rated for >200,000 x g

5.3.13. Ultracentrifuge

5.3.14. Water Bath

6. HEALTH AND SAFETY CONSIDERATIONS

6.1. Proper safety precautions should be taken while working in a laboratory setting. This includes, but is not limited to, proper protective equipment such as lab coats, safety glasses, closed-toe shoes, and non-latex gloves.

6.2. Refer to the respective SDS when working with any chemicals.

6.3. Refer to "HSL_GL_001: Waste Disposal at the Advanced Technology Research Facility" regarding waste disposal processes at the ATRF.

7. DEFINITIONS

Term	Definition
ATRF	Advanced Technology Research Facility
HPV	Human Papillomavirus
HSL	HPV Serology Laboratory
PEI	Polyethylenimine
SDS	Safety Data Sheets
SOP	Standard Operating Procedure
Type II water	Pure/Analytical Grade, used for standard applications

8. REAGENT PREPARATION

8.1.1. Transfection Lysis Buffer (15 mL)

8.1.1.1. Combine the following reagents:

- 13.255 mL of DPBS_MgCl_AA.
- 1 mL of 10% Brij58
- 62.5 µL of Benzonase
- 62.5 µL of Plasmid-Safe DNase
- 625 µL 1M Ammonium Sulfate, pH 9.0

8.1.1.2. Label with reagent name, current date, and analyst's initials.

8.1.1.3. Prepare reagent prior to use and maintain on wet ice or at 2-8°C.

9. 293TT CELL PREPARATION (DAY 1)

Note: Enter pertinent information on "HSL_LAB_005.01: HEK293TT Transfection Form, Day 1-4."

9.1. For T225 Cell Culture Flasks

9.1.1. Refer to "HSL_LAB_01: 293TT Cell Culturing and Maintenance" for information regarding the harvesting, counting, and seeding of cells.

9.1.2. Seed 21 x 10^6 293TT cells per flask in DMEM 10A in a total volume of 30 mL.

9.1.3. Incubate cells overnight (16-18 hours) in a 37°C, 5% CO_2 incubator.

9.2. For 5-Layer Cell Culture Flasks

9.2.1. Refer to "HSL_LAB_01: 293TT Cell Culturing and Maintenance" for information regarding the harvesting, counting, and seeding of cells.

9.2.2. Seed 84 x 10^6 cells per flask in DMEM 10A in a total volume of 120 mL.

9.2.3. Incubate cells overnight (16-18 hours) in a 37°C, 5% CO_2 incubator.

9.3. For 8-Layer CELLdisk Culture Flasks

9.3.1. Refer to "HSL_LAB_01: 293TT Cell Culturing and Maintenance" for information regarding the harvesting, counting, and seeding of cells.

9.3.2. Seed 189 x 10^6 cells per flask in DMEM 10A in a total volume of 270 mL.

9.3.3. Incubate cells overnight (16-18 hours) in a 37°C, 5% CO_2 incubator.

10. TRANSFECTION (DAY 2)

10.1. Confirm the 293TT cells are 40-60% confluent via inverted microscope.

Note: If confluency is below 40%, allow cells to grow until appropriate confluency has been reached.

10.2. Thaw HPV plasmid DNA on ice then mix by tapping gently on the side of the vial.

10.3. Confirm the concentration of the DNA using the NanoDrop1000. Refer to "HSL_EQ_021: Use and Maintenance of Nanodrop 1000 Spectrophotometer" for instruction on using the instrument.

10.3.1. Save NanoDrop1000 file as follows.

Transfection Logbook Number and Page where result is recorded
For example, *PDN2017099P002*

10.4. Prepare the Transfection Reagent:Opti-MEM mixture as shown in Table 1.

Note: "Transfection Reagent" can refer to either Lipofectamine 2000, PEI, or Transporter 5. Volumes and ratios are the same.

Table 1. Transfection Reagent:Opti-MEM ratio volumes

Flask Type	Transfection Reagent (per Flask)	Opti-MEM (per Flask)
T225	247.5 µL	5.625 mL
5-Layer	990 µL	22.5 mL
8-Layer CELLdisk	2228 µL	50.625 mL

10.4.1. Using Table 1 for guidance, combine Transfection Reagent and Opti-MEM as per the flask type being used and multiply the ratio by the total number of flasks being transfected.

Example:
Transfect 20 T225 Flasks;
Combine 4.95 mL Transfection Reagent with 112.5 mL Opti-MEM in a T75 Flask or bottle.

10.4.2. Incubate the Transfection Reagent:Opti-MEM mixture for 5-10 minutes at room temperature. Do not allow the Transfection Reagent to sit in Opti-MEM longer than 25 minutes.

10.4.3. Prepare the DNA:Opti-MEM mixture as shown in Table 2.

Table 2. DNA:Opti-MEM ratio volumes

Flask Type	DNA (per Flask)	Opti-MEM (per Flask)
T225	112.5 µg	5.625 mL
5-Layer	450 µg	22.5 mL
8-Layer CELLdisk	1013 µg	50.625 mL

10.4.4. Using a 1000 µL pipet, add the correct concentrations of DNA to Opti-MEM and gently mix by inverting the tube several times.

Example:
Transfect 20 T225 Flasks
Combine 2250 µg DNA with 112.5 mL Opti-MEM in a T75 Flask

10.5. Add the Transfection Reagent:Opti-MEM mixture to the DNA:Opti-MEM mixture into the appropriate-sized flask or bottle.

10.6. Incubate at room temperature for 20-30 minutes.

Note: Gently mix DNA:Transfection Reagent:Opti-MEM complex prior to adding into the flask.

10.7. Post-incubation, remove the 293TT media from the cells and add the DNA:Transfection Reagent:Opti-MEM complex directly to the 293TT flask. See Table 3 for the volume of complex to be added per type of culture flask.

Note: Gently mix the DNA:Transfection Reagent:Opti-MEM complex via rocking the cell flask back and forth to ensure even distribution of complex over cells.

Table 3. DNA:Transfection Reagent:Opti-MEM complex to be added after incubation

Flask Type	DNA:Transfection Reagent:Opti-MEM complex (per Flask)
T225	11.5 mL
5-Layer	46.0 mL
8-Layer CELLdisk	103.5 mL

10.8. Incubate the cells in a 37°C, 5% CO_2 incubator for 5-6 hours.

10.9. After incubation, remove the media via serological pipet or by decanting into a waste container and add 45 mL room-temperature, fresh DMEM 10A to each T225, 180 mL to each 5-layer flask, or 405 mL to each 8-layer flask.

10.10. Place the transfected cells in the 37°C, 5% CO_2 incubator for 48±2 hours.

11. CELL HARVEST (DAY 4)

11.1. Remove the cell supernatant containing media via serological pipet or by decanting into an appropriately-sized container “A”.

11.2. Gently wash attached cells with 15 mL PBS per each T225 flask, 40 mL for each 5-layer flask, or 75 mL for each 8-layer flask and collect wash into an appropriately-sized centrifuge container “B” (e.g. 50 mL or 500 mL conical).

Note: Depending on the confluency of the cells, post-transfection, increased wash volume or an additional wash may be necessary in order to remove media/serum from cells prior to trypsinization.

11.3. Trypsinize the cells in the flasks for 3-5 minutes in the 37°C, 5% CO_2 incubator using either 3 mL of Trypsin-EDTA for the T225 flasks, 15 mL of Trypsin-EDTA for the 5-layer flasks, or 30 mL for each 8-layer flask.

11.4. Post-trypsinization, collect detached cells into container “B”, then flush the flasks with the supernatant media from container “A” to remove any cells that are potentially still attached. Visually confirm that cells have detached from the flask.

11.5. Repeat procedure for additional flasks, washing with supernatant in container “A” in lieu of PBS.

11.6. Collect trypsinized cells and place into the same centrifuge conical used in section 11.2; if needed use an additional conical container. See Table 4 for amount of supernatant media to be added.

Note: The purpose of this procedure is to collect all 293TT cells, whether floating or adherent, to harvest all possible VLPs produced. The number of conical tubes will differ depending on the number of flasks and the volume of supernatant/wash/trypsinized cells. If cells are still in the flasks after the initial wash, wash the flasks one additional time with PBS/supernatant from container "A" with the volumes described and collect.

Table 4. Volume of supernatant media needed

Flask Type	Supernatant/ Media
T225	10 mL
5-Layer	40 mL
8-Layer CELLdisk	75 mL

11.7. Spin the conical tubes at 300 x g for 10 minutes at 20°C using the Sorvall Legend XTR centrifuge (Refer to SOP "HSL_EQ_003: Use and Maintenance of the Thermo Fisher Sorvall Legend XTR Centrifuge in the HPV Serology Laboratory").

11.8. Decant the media and add 5 or 15 mL of DPBS to the cell pellet, depending on which type of conical tube is being used (5 mL/50 mL conical or 15 mL/500 mL conical). Gently resuspend the cells via serological pipet.

11.9. Wash the cells via centrifugation at 300 x g for 10 minutes at 20°C using the Sorvall Legend XTR centrifuge.

11.10. Decant the supernatant and make sure any residual fluid is removed via pipet or absorption on an absorbent towel by inverting the tube and allowing all fluid to be collected upside down.

Note: The pellet will not be strongly adherent to the tube so make sure the pellet does not slide down onto the absorbent towel.

11.11. Add 1.5 times the cell-pellet volume with Transfection Lysis Buffer.

Note: Estimate the volume of the pellet by comparing to fluid in a dummy tube and add 1.5 volume of Transfection Lysis Buffer to the pellet.

11.12. Gently mix the cell pellet and lysis buffer mixture by tapping the side of the conical or via serological pipette.

11.13. Transfer 1 mL of cells resuspended in Transfection Lysis Buffer to a 1.5 mL siliconized tube.

11.14. Wrap parafilm around the lid of the siliconized tube and incubate the tubes for 22-26 hours in a 37±2°C water bath to allow for VLP maturation. Invert the tubes 1-2 times in the first two hours of incubation to ensure uniform lysis and exposure to lysate reagents.

Note: Some HPV types may need a longer maturation.

11.15. Following maturation, transfer the tubes to wet ice or to a 2-8°C refrigerator for 10 minutes. Post-incubation, add 0.175 volumes of 5M NaCl (0.175 mL/mL cell lysate) to the lysate and gently mix by tapping the tube or inverting 3-5 times. Next, incubate the mixture for 10 minutes on wet ice or in a 2-8°C refrigerator.

11.16. Freeze vials at -80°C to be used for future purification.

Note: Alternately, lysates can be purified and collected via gradient on the same day they are prepared by performing the following steps.

11.16.1. Chill lysate on ice for 10-15 minutes, add 0.175 mL 5M NaCl, and then perform one freeze-thaw cycle by storing the lysate at -80°C for at least one hour and then thaw on ice. During this incubation, prepare gradient as described in Section 12.

12. GRADIENT AND PURIFICATION (DAY 5)

Note: Enter pertinent information on "HSL_LAB_005.02: HEK293TT Transfection Form, Day 5."

12.1. Ultracentrifuge Preparation

12.1.1. Turn on the ultracentrifuge and prepare it for use.

12.1.1.1. Select program per Table 1; Confirm settings for rotor, speed and temperature.

12.1.1.2. Select rotor type. Confirm proper tube size. See Table 1 for proper selection.

Table 1: Gradients

Program Name	Rotor Type	Tube P/N	Volume of Each Gradient to Use (µL)	Rotor Speed	Length of time (hour : min)
HPV_PsV	SW 55 Ti	326819	700	303,800 x g	03:30
HPV_PsV_SW40	SW40 1 Ti	331374	1400	284,600 x g	04:45

12.1.1.3. Place rotor in the ultracentrifuge.

12.1.1.4. Close the ultracentrifuge lid and confirm the vacuum seal is working properly.

12.1.1.5. Set ultracentrifuge to 16°C and allow 30-60 minutes prior to use for cool down (HSL_EQ_024).

12.2. Gradient Preparation

12.2.1. Pour a 27%, 33%, and 39% Opti-Prep step gradient into Thinwall Polypropylene Tubes, using volumes appropriate to tube size and rotor being used, per Table 1.

12.2.2. Using a sterile syringe fitted with a cannula, add 27% Opti-Prep to the bottom of the tube.

12.2.3. Using a clean syringe fitted with a clean cannula, underlay 33% Opti-Prep by slowly dispensing until the entire volume is in the tube.

12.2.4. Using a clean syringe fitted with a clean cannula, underlay 39% Opti-Prep by slowly dispensing until the entire volume is in the tube.

Note: When held at eye level in the BSC, an interface between gradients should be visible.

Note: Rinse the cannulas 10x with Type II water following procedure to prevent the Opti-prep from clogging the cannula for future use.

12.2.5. Allow gradient to diffuse 1-2 hours at room temperature with minimal light exposure.

12.2.6. While the gradient is diffusing, remove the lysates from freezer and allow them to thaw on wet ice. Once lysates are completely thawed, invert tubes gently to mix and remove parafilm if present.

12.2.7. Clarify the lysate by centrifuging at 10000 x g at 4°C for 10 minutes.

Note: More than one centrifuge clarification may need to be completed in order to fully pellet the cell debris from the lysate.

12.2.8. Remove the clarified supernatant from the tube, and transfer it to a 1.5 mL siliconized tube, and store the tube with supernatant on wet ice.

12.2.9. Add 400 µL of DPBS_0.8M to the cell pellet(s) post-clarification. Gently mix each tube using a pipette, then centrifuge at 10000 x g at 4°C for 10 minutes.

Note: A significant amount of VLPs may still be found in the pellet and washing the pellet ensures that most VLPs have been collected.
Note: Term this step “Cell Wash”, and keep this supernatant separate from the primary lysate supernatant loaded on the Opti-Prep gradients.

12.2.10. Remove the clarified supernatant from the tube, and transfer it to a 1.5 mL siliconized tube, and store the tube with supernatant on wet ice.

12.2.11. Store supernatants on wet ice until gradient has fully diffused.

12.3. Ultracentrifugation

12.3.1. Carefully add the collected supernatant to the top of the Opti-Prep gradient using a siliconized pipet tip. Pipette the supernatant slowly so that the gradient is not disturbed.

Note: Keep lysate types together, this includes using a single ultracentrifuge tube for the lysates washed with DPBS_0.8M buffer.

12.3.2. Fill each gradient tube until it is approximately 4 mm from the top of the tube to prevent collapse during the ultracentrifugation step, then slowly place the gradient tube into the ultracentrifuge bucket.

12.3.3. Using a Precision Balance, place the first bucket with tube onto the balance then press “Tare”. Next, place the corresponding bucket with tube on the balance.

12.3.4. Using DPBS_0.8M, adjust the volume of the bucket with tube with the lower weight until they are equal. Repeat for the remaining bucket with tubes (bucket with tube pairing: 1&4, 2&5, 3&6).

12.3.5. Tightly screw the bucket lid closed.

12.3.6. Load the buckets on to the rotor and verify that the buckets swing freely.

12.3.7. Load the rotor into the ultracentrifuge, and select the appropriate program corresponding to Table 1.

Note: To avoid disturbing the gradient, minimal brake is used so it takes approximately 30 minutes for the ultracentrifuge to stop.

12.3.8. Once the program has completed, carefully remove the rotor from the ultracentrifuge.

12.4. Gradient Collection

12.4.1. Post-ultracentrifugation, collect fractions from the bottom of the tubes by securing the tube with clamp and stand. Once secure, carefully pierce a hole in the bottom of the Thinwall Polypropylene Tube with a 25G needle.

Note: Wear protective, puncture-resistant gloves when working with needles.

12.4.2. Fractions are collected at the following volumes in labeled siliconized 1.5 mL tubes (see Attachment 1 for labeling guidance).

Rotor Type	Tube P/N	Volume of Fraction 1 to Collect (µL)	Volume of Fractions 2-10 to Collect (µL)
SW 55 Ti	326819	400	200
SW40 1 Ti	331374	1000	300

12.4.2.1. Gently mix the fractions (do not vortex) and aliquot approximately 20 µL of each fraction for confirmatory testing.

12.4.2.2. Store VLPs at -80°C in a properly labeled box (see Attachment 1 for labeling guidance).

Note: Store 20 µL aliquots in a separate box.

Attachment 1: Label Guidance

Study: *STD 24 hr HPV16shell Mature Lysate*
Sample Type: *293TT Cell Lysate*
Date: *19JUN17*
Initials: *TK*
Box *1* of *2*

293TT Lysate
VLP L1L2 HPV16
20x5Layer
RIPCORD 1M NH3SO4
Cell Pellet (1:1.5)
02JUN17 CA

Study: *HPV16shell Ultratube # XX-YY*
Sample Type: *OptiPrep*
Date: *19JUN17*
Initials: *TK*
Box *1* of *2*

OptiPrep
VLP L1L2 HPV16
Fraction#10
80-T225 Flask
14JUN17 TK Code I
UltraTube: # 56

13. REVISION HISTORY

Revision Start Date	Version #	Changes	Reasons
26Apr17	New	Create new SOP describing the transfection of HEK293TT for VLP production and purification.	New SOP.
26Jul17	1.0	Add Table 1 to update Rotor Information and related cycle speeds/time. Update sections of the SOP to reflect those changes.	Increase rate of production of VLP.

Equipment Name	Equipment ID	Calibration Due Date
BSC	☐ HSL_007 ☐ HSL_008 ☐ HSL_009 ☐Other:	
37°C Incubator	☐ HSL_024 ☐ HSL_023 ☐ HSL_026 ☐ HSL_027 ☐Other:	
Microscope	☐ HSL_020 ☐Other:	☐N/A
NanoDrop	☐ HSL_036 ☐Other:	
Sorvall Legend XTR	☐ HSL_033 ☐Other:	
Water Bath	☐ HSL_010 ☐Other:	☐N/A
-80°C Freezer	☐ HSL_022 ☐Other:	
2-8°C Refrigerator	☐ HSL_029 ☐Other:	
☐N/A Pipette: µL		
☐N/A Pipette: µL		

Reagent Name	Lot Number	Expiration Date
DPBS_MgCl_AA		
10% Brij58		
Benzonase		
Plasmid-Safe DNase		
1M Ammonium Sulfate, pH 9.0		
DMEM 10A		
Transfection Reagent:		
Opti-MEM		
DPBS		
Trypsin-EDTA		

Comments:

☐N/A

Performed By/ Date:	
Reviewed By/ Date:	

Cell Preparation

Cell Line Lot#/ Passage #:	Type of Flask/ # Prepared:

DNA Used/ NanoDrop Results

Description/Lot#	
Concentration	

Transfection Reagent:Opti-MEM	Volume Used (mL)
Transfection Reagent	
Opti-MEM	

DNA:Opti-MEM	Volume Used (mL)
DNA	
Opti-MEM	

Incubate at RT for ≥ 5 minutes

Incubate DNA: Lipofectamine: Opti-MEM for 20-30 minutes at RT

Addition of DNA:Lipo:Opti-MEM

Volume Added/Flask:_____

First Flask to Final Flask Start Time:_____ End Time:_____
(For Information Only)

Incubate the cells in a 37°C, 5% CO2 incubator for 5-6 hours

Place the transfected cells in a 37°C, 5% CO_2 incubator for 48±2 hours

VLP Maturation

Incubate the tubes in a 37°C water bath (type-specific)

Date/Start Time: _____ Date/End Time: _____

Incubate the lysate for 10±2 minutes at 2-8°C

Add 0.175 mL of 5M NaCl per 1 mL lysate

Incubate the lysate for 10±2 minutes at 2-8°C

Store at -80°C

Comments:

☐N/A

Performed By/ Date:	
Reviewed By/ Date:	

Equipment Name	Equipment ID	Calibration Due Date
BSC	☐ HSL_007 ☐ HSL_008 ☐ HSL_009 ☐Other:	
Eppendorf Centrifuge	☐ HSL_006 ☐Other:	
Precision Balance	☐ HSL_015 ☐Other:	
Ultracentrifuge	☐ HSL_001 ☐Other:	
-80°C Freezer	☐ HSL_022 ☐Other:	
2-8°C Refrigerator	☐ HSL_029 ☐Other:	
Rotor Used	☐ Sw 55 Ti ☐ Sw40 1 Ti	**N/A**
☐N/A Pipette: µL		
☐N/A Pipette: µL		

Reagent Name	Lot Number	Expiration Date
27% OptiPrep		
33% OptiPrep		
39% OptiPrep		
DPBS_0.8M		

Ultra-Centrifuge Tube Position	Ultra-Centrifuge Sequence #	Data Reference/Description of Lysate **Note**: If same lysate is used for multiple lines, write "Same as Tube X" where "X" is the Ultra-Centrifuge Tube #
☐N/A 1		
☐N/A 2		
☐N/A 3		
☐N/A 4		
☐N/A 5		
☐N/A 6		

	Start Time	End Time
Gradient Diffusion (For Information Only)		

Comments:

☐N/A

Performed By/ Date:	
Reviewed By/ Date:	